Exhibit 1

to Applicant's response of 30 November 2010 in USSN 09/445,517

Sustained Reduction in Food Intake and Body Weight in High Fat-Fed Rats During 28-Day Amylin Infusion

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Abstract

The β-cell hormone amylin exhibits anorexigenic actions in a number of acute rodent feeding models. In insulin-treated patients with type 1 and type 2 diabetes, adjunctive treatment with pramlintide, a synthetic amylin analog, leads to a decrease in HbA_{1c} that is accompanied by a sustained reduction in body weight. To further assess the long-term effects of amylin on food intake and body weight, we compared the effects of 28 d amylin (300 µg/kg/day) and sibutramine infusion (3 mg/kg/day) in high fat-fed rats. Subjects were placed on a high fat (60% kcal from fat) or low fat diet (10% kcal from fat) for ten weeks; high fat-fed rats were then implanted s.c. with osmotic pumps containing amylin (n = 10) or sibutramine (n = 10). The remaining high fat-fed (n = 12) and the low fat-fed rats (n = 8) served as controls (body weight prior to drug treatment: high fat = 531 + 5 g, low fat = 496 + 9 g [mean + SEM]). Analysis by week showed sustained reductions with amylin, but not sibutramine treatment, across the 4-week treatment period (percent decrease compared to high fat-fed controls):

	Amylin		Sibutramine	
Week	**Food Intake**	**Body Weight**	**Food Intake**	**Body Weight**
1	**45%***	**6%***	**45%***	**6%***
2	**14%***	**7%***	**8%***	**6%***
3	**10%***	**8%***	**-1%**	**6%***
4	**10%***	**8%***	**-3%**	**3%**

* P<0.05

Body weight after 28 d drug infusion was 513 + 10 g for amylin (P<0.002 vs. controls), 540 + 11 g for sibutramine (P = 0.17 vs. controls), 558 + 7 g for high fat controls and 532 + 9 g for low fat controls. Body composition analysis showed both amylin and sibutramine treatment reversed the increase in body fat observed in high fat-fed animals. Amylin treatment reduced plasma leptin and insulin levels, while sibutramine reduced plasma insulin levels. A second amylin dose-response study showed significant body weight reduction at 28 d at a dose of 30 µg/kg/day. These data indicate that both the anorexigenic and weight-reducing effects of amylin are maintained with sustained drug exposure, contrasting the effects observed with sibutramine in which tolerance developed during the second half of the drug exposure period.

Note: Abstract is updated to reflect additional studies.

Introduction

Amylin is a β-cell hormone secreted in coordination with insulin in response to carbohydrate (glucose) and protein-derived amino acids following a meal stimulus. In clinical trials in insulin-treated patients with type 1 and type 2 diabetes, adjunctive treatment with pramlintide, a synthetic amylin analog, leads to a decrease in HbA_{1c} that is accompanied by a reduction in body weight. To further explore the weight loss effects of amylin, energy-related measures were obtained in high fat-fed rats treated for 28 days with amylin. Another group of animals received sibutramine for comparison. In a second study, the dose-response effect of amylin in this model was explored.

Methods

Study 1. High fat-fed, male Sprague-Dawley rats were implanted subcutaneously with 28-day osmotic pumps (Durect Corp.) delivering amylin (300 µg/kg/day), sibutramine (3 mg/kg/day), or vehicle (50% DMSO).

Study 2. High fat-fed rats were implanted with three doses of amylin (30, 100, and 300 µg/kg/day) or vehicle.

Low fat-fed animals in both studies received vehicle pumps. Diets were purchased from Research Diets (D12331, 58% kcal from fat, and D12329, 11% kcal from fat). Food intake and body weight measurements were obtained weekly. Rats were sacrificed by cardiac puncture under anesthesia and metabolic indicators measured on a COBAS Mira plasma analyzer (Roche). Body composition was measured by chemical analysis (Covance Laboratories, Madison, WI).

Amylin was synthesized at Amylin Pharmaceuticals, Inc. by solid-phase chemistry, purified by HPLC (>98% purity, 84% peptide content), and characterized by amino acid analysis and LC/MS. Sibutramine was extracted from the drug product MERIDIA® using water as a solvent, purified by RP-HPLC (>98% purity), and characterized by NMR and LC/MS.

All data are represented as mean ± SEM. Analysis of variance was used to test for group differences.

Results

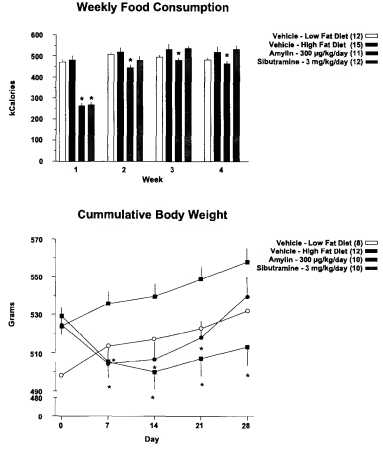
During the 10-week fattening period in Study 1, high fat-fed rats were designated as obesity-prone (top 50%) or obesity-resistant (bottom 50%) based on the amount of weight gained through Week 71. No differences between prone and resistant animals were observed for food consumption, body weight, or plasma metabolites in response to drug treatment; therefore, these groups were combined (Figures 1 and 3). An obesity-prone/resistant X drug interaction was found for protein weight in amylin-treated rats, and thus body composition parameters were measured separately in obesity-prone and obesity-resistant animals for each drug group (Figure 2).

Table 1. % Reduction in Food Consumption and Body Weight During Sustained Exposure to Amylin or Sibutramine

	Weekly Caloric Intake				Cummulative Body Weight			
Week	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Study 1								
Amylin 300 µg/kg/day	45*	14*	10*	10*	6*	7*	8*	8*
Sibutramine 3 mg/kg/day	45*	8*	-1	-3	6*	6*	6*	3
Study 2								
Amylin								
30 µg/kg/day	32*	17*	10	8	5*	7*	7*	7*
100 µg/kg/day	45*	19*	4	9	10*	10*	9*	8*
300 µg/kg/day	41*	15*	19*	19*	7*	8*	10*	12*

* Significantly different from high fat-fed controls.

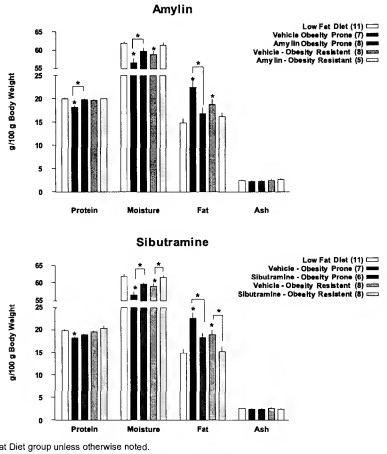
Figure 1. Amylin, but not Sibutramine, Produces Sustained Decreases in Food Consumption and Body Weight Gain During Chronic Infusion



* P< 0.05 compared to Vehicle - High Fat Diet group.

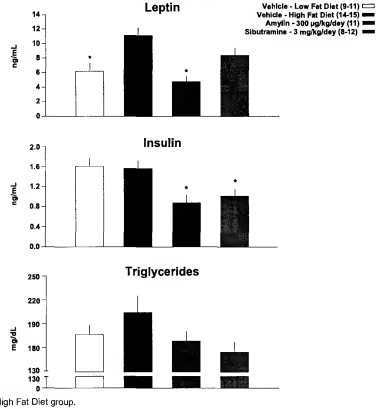
Results

Figure 2. Both Amylin and Sibutramine Prevent the Increase in Body Fat Induced by a High Fat Diet



* P<0.05 compared to the Low Fat Diet group unless otherwise noted.

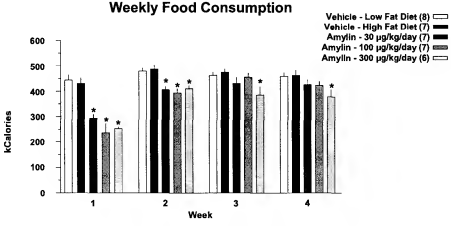
Figure 3. Amylin Reduces Leptin and Insulin Levels; Sibutramine Reduces Insulin Levels

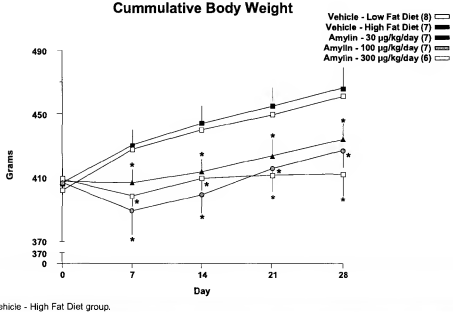


* P<0.05 compared to Vehicle - High Fat Diet group.

Results

Figure 4. Amylin dose-dependently decreases food consumption and body weight gain, with a significant reduction in body weight gain observed at 30 µg/kg/day





* P<0.05 compared to Vehicle - High Fat Diet group.

Conclusions

- Four-week exposure to amylin, but not sibutramine, produces sustained reductions in food consumption and body weight gain in high fat-fed rats.
- Both amylin and sibutramine prevent the increased body fat content induced by a high fat diet.
- The effects of amylin on food intake and body weight gain are dose-dependent, with a reduction in body weight gain observed at 30 µg/kg/day.
- The data support an effect of amylin in the regulation of food intake, body weight, and body composition in a clinically relevant model of obesity.

References

1. Levin BE, Dunn-Meynell AA. Defense of body weight against chronic caloric restriction in obesity-prone and -resistant rats. Am J Physiol Regul Integr Comp Physiol. 2000; 278(1):R231-237.